

**REMARKS**

Claims 19-24, 27-31, and 33-38 are now pending, with Claim 19 being the sole independent claim.

Claims 25, 25, and 32 have been canceled without prejudice to or disclaimer of the subject matter recited therein.

Claims 19-24, 27, 33, and 35 have been amended. Claims 36-38 have been added. Support for these claim amendments are found throughout the specification, and at least at page 13, line 29; and Example 7. No new matter has been added.

The amendments to the specification merely correct clerical errors and remove hyperlinks to the world wide web. These changes are not believed to add any new matter to the application.

**RESPONSE TO RESTRICTION REQUIREMENT**

In the Office Action, Claims 19-35 were subject to restriction and/or election requirement. Applicants hereby elect Group I invention G without traverse.

Pending claims 19-24, 27-31, and 33-38 are directed to the elected invention of Group I invention G.

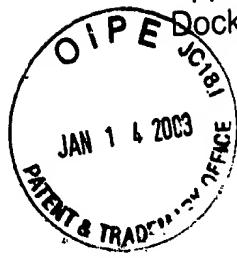
Please charge any requisite fees or credit any overpayment to Deposit Account No. 04-1928 (E. I. du Pont de Nemours and Company).

In view of the foregoing, allowance of the application is earnestly solicited.

Respectfully submitted,



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**MARKED-UP VERSION SHOWING CHANGES MADE**

In showing changes made, deletions are shown in [brackets with strikethrough], and additions are underlined.

**IN THE SPECIFICATION:**

**Paragraph at page 1, line 31- page 2, line 4:**

It is preferred that the isolated polynucleotides of the claimed invention consists of a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, and 13 that codes for the polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, and 14. The present invention also relates to an isolated polynucleotide comprising a nucleotide sequences of at least [one of]60 (preferably at least [one of]40, most preferably at least [one of]30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, and 13 and the complement of such nucleotide sequences.

**Paragraph at page 2, lines 5-7:**

The present invention relates to an isolated polynucleotide comprising at least [one of]30 contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13 and the complement of such sequences.

**Paragraph at page 2, line 36 to page 3, line 7:**

The present invention relates to a method of obtaining a nucleic acid fragment encoding a substantial portion of a lecithin:cholesterol acyltransferases polypeptide gene, preferably a plant plant lecithin:cholesterol acyltransferases polypeptide gene, comprising the steps of: synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least [one of]60 (preferably at least [one of]40, most preferably at least [one of]30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, and 13 and the complement of such nucleotide sequences; and amplifying a nucleic acid fragment (preferably a cDNA inserted in a cloning vector) using the oligonucleotide primer. The amplified nucleic acid fragment preferably will encode a portion of a plant lecithin:cholesterol acyltransferases amino acid sequence.

**Paragraph at page 4, line 22 to page 5, line 5:**

In the context of this disclosure, a number of terms shall be utilized. As used

that optionally contains synthetic, non-natural or altered nucleotide bases. A polynucleotide in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA, synthetic DNA, or mixtures thereof. An isolated polynucleotide of the present invention may include at least [one of] 60 contiguous nucleotides, preferably at least [one of] 40 contiguous nucleotides, most preferably [one of] at least 30 contiguous nucleotides, of the nucleic acid sequence of the SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, or the complement of such sequences.

**Paragraph at page 5, lines 26-36:**

Substantially similar nucleic acid fragments may be selected by screening nucleic acid fragments representing subfragments or modifications of the nucleic acid fragments of the instant invention, wherein one or more nucleotides are substituted, deleted and/or inserted, for their ability to affect the level of the polypeptide encoded by the unmodified nucleic acid fragment in a plant or plant cell. For example, a substantially similar nucleic acid fragment representing at least [one of] 30 contiguous nucleotides derived from the instant nucleic acid fragment can be constructed and introduced into a plant or plant cell. The level of the polypeptide encoded by the unmodified nucleic acid fragment present in a plant or plant cell exposed to the substantially similar nucleic fragment can then be compared to the level of the polypeptide in a plant or plant cell that is not exposed to the substantially similar nucleic acid fragment.

**Paragraph at page 5, line 37 to page 6, line 28:**

For example, it is well known in the art that antisense suppression and co-suppression of gene expression may be accomplished using nucleic acid fragments representing less than the entire coding region of a gene, and by nucleic acid fragments that do not share 100% sequence identity with the gene to be suppressed. Moreover, alterations in a nucleic acid fragment which result in the production of a chemically equivalent amino acid at a given site, but do not effect the functional properties of the encoded polypeptide, are well known in the art. Thus, a codon for the amino acid alanine, a hydrophobic amino acid, may be substituted by a codon encoding another less hydrophobic residue, such as glycine, or a more hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for another, such as aspartic acid for glutamic acid, or one positively charged residue for another, such as lysine for arginine, can also be expected to produce a functionally equivalent product. Nucleotide changes which result in alteration of the N-terminal and C-terminal portions of the polypeptide molecule would also not be expected to alter the activity of the polypeptide. Each of the above modifications is well within the routine skill of the artisan. Each of the above modifications is well within the routine skill of the artisan.

products. Consequently, an isolated polynucleotide comprising a nucleotide sequence of at least [one of] 60 (preferably at least [one of] 40, most preferably at least [one of] 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13 and the complement of such nucleotide sequences may be used in methods of selecting an isolated polynucleotide that affects the expression of a polypeptide (such as lecithin:cholesterol acyltransferases) in a host cell. A method of selecting an isolated polynucleotide that affects the level of expression of a polypeptide in a host cell (eukaryotic, such as plant or yeast, prokaryotic such as bacterial, or viral) may comprise the steps of: constructing an isolated polynucleotide of the present invention or an isolated chimeric gene of the present invention; introducing the isolated polynucleotide or the isolated chimeric gene into a host cell; measuring the level a polypeptide in the host cell containing the isolated polynucleotide; and comparing the level of a polypeptide in the host cell containing the isolated polynucleotide with the level of a polypeptide in a host cell that does not contain the isolated polynucleotide.

**Paragraph at page 7, line 27 to page 8, line 11:**

A "substantial portion" of an amino acid or nucleotide sequence comprises an amino acid or a nucleotide sequence that is sufficient to afford putative identification of the protein or gene that the amino acid or nucleotide sequence comprises. Amino acid and nucleotide sequences can be evaluated either manually by one skilled in the art, or by using computer-based sequence comparison and identification tools that employ algorithms such as BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol.* 215:403-410[; see also [www.ncbi.nlm.nih.gov/BLAST/](http://www.ncbi.nlm.nih.gov/BLAST/)]). In general, a sequence of ten or more contiguous amino acids or thirty or more contiguous nucleotides is necessary in order to putatively identify a polypeptide or nucleic acid sequence as homologous to a known protein or gene. Moreover, with respect to nucleotide sequences, gene-specific oligonucleotide probes comprising 30 or more contiguous nucleotides may be used in sequence-dependent methods of gene identification (e.g., Southern hybridization) and isolation (e.g., *in situ* hybridization of bacterial colonies or bacteriophage plaques). In addition, short oligonucleotides of 12 or more nucleotides may be used as amplification primers in PCR in order to obtain a particular nucleic acid fragment comprising the primers. Accordingly, a "substantial portion" of a nucleotide sequence comprises a nucleotide sequence that will afford specific identification and/or isolation of a nucleic acid fragment comprising the sequence. The instant specification teaches amino acid and nucleotide sequences encoding polypeptides that comprise one or more particular plant proteins. The skilled artisan, having the benefit of the sequences as

reported herein, may now use all or a substantial portion of the disclosed sequences for purposes known to those skilled in this art. Accordingly, the instant invention comprises the complete sequences as reported in the accompanying Sequence Listing, as well as substantial portions of those sequences as defined above.

**Paragraph at page 12, line 14 to page 13, line 6:**

In addition, two short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols to amplify longer nucleic acid fragments encoding homologous genes from DNA or RNA. The polymerase chain reaction may also be performed on a library of cloned nucleic acid fragments wherein the sequence of one primer is derived from the instant nucleic acid fragments, and the sequence of the other primer takes advantage of the presence of the polyadenylic acid tracts to the 3' end of the mRNA precursor encoding plant genes. Alternatively, the second primer sequence may be based upon sequences derived from the cloning vector. For example, the skilled artisan can follow the RACE protocol (Frohman et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:8998-9002) to generate cDNAs by using PCR to amplify copies of the region between a single point in the transcript and the 3' or 5' end. Primers oriented in the 3' and 5' directions can be designed from the instant sequences. Using commercially available 3' RACE or 5' RACE systems (BRL), specific 3' or 5' cDNA fragments can be isolated (Ohara et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:5673-5677; Loh et al. (1989) *Science* 243:217-220). Products generated by the 3' and 5' RACE procedures can be combined to generate full-length cDNAs (Frohman and Martin (1989) *Techniques* 1:165). Consequently, a polynucleotide comprising a nucleotide sequence of at least [one of] 60 (preferably [one of] at least 40, most preferably [one of] at least 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, and the complement of such nucleotide sequences may be used in such methods to obtain a nucleic acid fragment encoding a substantial portion of an amino acid sequence of a polypeptide. The present invention relates to a method of obtaining a nucleic acid fragment encoding a substantial portion of a polypeptide of a gene (such as plant lecithin:cholesterol acyltransferases) preferably a substantial portion of a plant polypeptide of a gene, comprising the steps of: synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least [one of] 60 (preferably at least [one of] 40, most preferably at least [one of] 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, and 13, and the complement of such nucleotide sequences; and amplifying a nucleic acid fragment (preferably a cDNA insert) using the oligonucleotide primer. The amplified nucleic

**Paragraph at page 18, lin 27 to pag 19, line 15:**

cDNA clones encoding plant lecithin:cholesterol acyltransferases were identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol.* 215:403-410[; see also [www.ncbi.nlm.nih.gov/BLAST/](http://www.ncbi.nlm.nih.gov/BLAST/)]) searches for similarity to sequences contained in the BLAST "nr" database (comprising all non-redundant GenBank CDS translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank, the last major release of the SWISS-PROT protein sequence database, EMBL, and DDBJ databases). The cDNA sequences obtained in Example 1 were analyzed for similarity to all publicly available DNA sequences contained in the "nr" database using the BLASTN algorithm provided by the National Center for Biotechnology Information (NCBI). The DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein sequences contained in the "nr" database using the BLASTX algorithm (Gish and States (1993) *Nat. Genet.* 3:266-272) provided by the NCBI. For convenience, the P-value (probability) of observing a match of a cDNA sequence to a sequence contained in the searched databases merely by chance as calculated by BLAST are reported herein as "pLog" values, which represent the negative of the logarithm of the reported P-value. Accordingly, the greater the pLog value, the greater the likelihood that the cDNA sequence and the BLAST "hit" represent homologous proteins.

**IN THE CLAIMS:**

**Please amend claims 19, 20, 21, 22, 23, 24, 27, 33, and 35 as follows:**

19. (amended) An isolated polynucleotide comprising: (a) a nucleic sequence encoding a polypeptide having[that encodes a] plant lecithin:cholesterol acyltransferase[s polypeptide having a sequence identity] activity, wherein the polypeptide has an amino acid sequence of at least 80% sequence identity, based on the Clustal method of alignment, when compared to [a polypeptide selected from the group consisting of] SEQ ID NO[s: 2, 4, 6, 8, 10, 12 and ]:14; or (b) a complement of the nucleic acid sequence wherein the complement and the nucleic acid sequence consist of the same number of nucleotides and are 100% complementary.

20. (amended) The polynucleotide of Claim 19 wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:14 have[sequence identity is] at least 85% identity based on the Clustal alignment method.

21. (amended) The polynucleotide of Claim 19 wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:14

have[sequence identity is] at least 90% identity based on the Clustal alignment method.

22. (amended) The polynucleotide of Claim 19 wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:14 have[sequence identity is] at least 95% identity based on the Clustal alignment method.

23. (amended) The polynucleotide of Claim 19 wherein the polypeptide[~~is selected from the group consisting~~]comprises the amino acid sequence of SEQ ID [Nos:2, 4, 6, 8, 10, 12, and 14]NO:14.

24. (amended)The polynucleotide of Claim 19, wherein the polynucleotide [~~is selected from SEQ ID Nos:1, 3, 5, 7, 9, 11, and 13~~]comprises the nucleic acid sequence of SEQ ID NO:13.

27. (amended) A cell or a virus comprising the polynucleotide of Claim [19]34.

33. (amended) A chimeric gene comprising the polynucleotide of Claim 19 operably linked to at least one [suitable]regulatory sequence.

35. (amended) A method for altering the level of plant lecithin:cholesterol acyltransferases polypeptide expression in a host cell, the method comprising:

- a) Transforming a host cell with the chimeric gene of claim [60]34; and
- b) Growing the transformed cell in step (a) under conditions suitable for the expression of the chimeric gene.